Meeting of the National Toxicology Program (NTP) Advisory Committee on Alternative Toxicological Methods (ACATM)

September 25, 1998
Final Minutes
National Institute of Environmental Health Sciences (NIEHS)
Building 101
Research Triangle Park, NC

The following ACATM members were in attendance:

- Katherine Stitzel, D.V.M. (Chair), Procter & Gamble Company, Cincinnati, Ohio
- Paul Bailey, Ph.D., Mobil Business Resources Corporation, Paulsboro, New Jersey
- Michael Denison, Ph.D., University of California—Davis, Davis, California
- Alan Goldberg, Ph.D., Johns Hopkins University, Baltimore, Maryland
- Sidney Green, Ph.D., Covance Laboratories, Vienna, Virginia
- Susan Hurt, Ph.D., Rohm and Haas Company, Spring House, Pennsylvania
- Charles Montgomery, D.V.M., Baylor College of Medicine, Houston, Texas
- Andrew Rowan, Ph.D., Humane Society of the United States (HSUS), Gaithersburg, Maryland
- Peter Theran, D.V.M., Massachusetts Society for the Prevention of Cruelty to Animals, Boston, Massachusetts

The following ACATM members were absent:

- Elaine Faustman, Ph.D., University of Washington, Seattle, Washington
- A. Wallace Hayes, Ph.D., Gillette Company, Boston, Massachusetts
- Roger McClellan, D.V.M., Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina

Other Meeting Attendees:

Randy Allen, Hybrizyme
Carl Barrett, NIEHS
Rodney Boatman, Eastman Kodak
Loretta Brammell, NIEHS/NICEATM
John Bucher, NIEHS
Kathleen Cater, Dial Corporation
Raj Chhabra, NIEHS
George Clark, XDS
Terri Damstra, WHO/IPCS
Janis Demetrulias, Technikos Research
Carol Eisenmann, CTFA
Monica Frost, NIEHS
Frank Gerberick, Procter & Gamble
Karen Hamernik, EPA
Terry Hammond, BNA Publishers
Patrick Herron, ILS, Inc./NICEATM
Steven Kinsler, R.J. Reynolds Corporation
Sandy Lang, NIEHS
George Lucier, NIEHS
Tony Maciorowski, EPA
Debbie McCarley, NIEHS/NICEATM
Harry Salem, ERDEC
Doug Sharpnack, NIOSH
Dee Sailstad, EPA
Mary Jane Selgrade, EPA
Jim Stevens, Novartis
William Stokes, NIEHS/NICEATM
Raymond Tice, ILS, Inc./NICEATM
Heather Vahdat, ILS, Inc./NICEATM
Kathryn Valeda, NHLBI
Neil Wilcox, FDA
Errol Zeiger, NIEHS

Call to Order and Introductions
Dr. Stitzel called the meeting to order at 8:45 a.m. and asked all panel members and the audience to introduce themselves for the record.

Welcome
Dr. Carl Barrett, Director of the NIEHS Division of Intramural Research welcomed the panel and the audience. He emphasized the important role of the Committee in providing advice to the NIEHS on its activities and priorities relating to alternative test methods, particularly the ICCVAM and the NTP Center.

NTP Update
Dr. George Lucier, Director of the Environmental Toxicology Program (ETP), provided an update of the activities of the NTP. The National Toxicology Program, headquartered at NIEHS, established in 1978 to:
1) Provide toxicological evaluation on substances of public health concern
2) Develop and validate improved methods (sensitive-specific-faster)
3) Develop approaches and generate data to strengthen the science base for risk assessments
4) Communicate with all stakeholders

which directed the NIEHS to develop and gain regulatory acceptance of alternative toxicological methods is fully consistent with the goals of the NTP. Dr. Lucier reviewed the priority list of NTP initiatives, which included alternative methods.

Update on the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Activities
Dr. William Stokes, Director of NICEATM and ICCVAM Co-Chair, presented background information on ICCVAM and NICEATM. ICCVAM was established in May 1997, and consists of representatives from 14 Federal regulatory and research agencies and programs. NICEATM was established in April 1998 to provide operational support for ICCVAM, to organize test method peer reviews and workshops, and to serve as the point of contact for stakeholders. The goal of ICCVAM and NICEATM is to promote the scientific validation and regulatory acceptance of new alternative test methods that are more predictive of human and ecological effects than current methods. In achieving the goal, methods are sought that will also reduce, refine, and replace animal use whenever scientific feasible. Dr. Stokes reviewed the purpose of ICCVAM peer review meetings and workshops. The purpose of peer review meetings is to develop scientific consensus on the usefulness of proposed test methods for specific human health or ecological risk assessment purposes. The purpose of ICCVAM workshops is to: evaluate the adequacy of current methods for assessing specific toxicities; evaluate the current validation status of a test method or related methods; identify additional research, development, and validation studies needed; and to prioritize development and validation efforts or methods that should be further supported. Three test methods are currently being reviewed by ICCVAM in the future. These include the Murine Local Lymph Node Assay (LLNA), a method for assessing allergic contact dermatitis; Corrositex®, an *in vitro* test method for assessing dermal corrosivity test method; and the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX), a developmental toxicity/teratogenicity screening method. Dr. Stokes also mentioned methods that may be considered by ICCVAM in the future. These include the 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay, endocrine screening and testing methods, transgenic mouse models for carcinogenicity, the HCE-7 *in vitro* method for assessing ocular irritancy, and the Mouse Ear Swelling Test (MEST) for assessing allergic contact dermatitis. Dr. Stokes discussed the role of the ICCVAM Immunotoxicity Working Group (IWG) in

facilitating the LLNA peer review process and emphasized the importance of involving knowledgeable scientists from both ICCVAM regulatory and research agencies.

Dr. Green asked why ICCVAM was scheduling a peer review and workshop for FETAX as opposed to other developmental toxicity assays. Dr. Stokes responded that the Environmental Protection Agency (EPA) had requested that ICCVAM review the validation status of FETAX. A member commented that FETAX offered a wider range of applicability because of its potential for both human health and ecological hazard assessments. Dr. Stitzel asked if there had been a formal validation of FETAX. Dr. Stokes replied that a validation study had been conducted in partnership between NIEHS and the Department of Defense (DoD).

In regard to humane endpoints for testing methods, Dr. Goldberg asked if there were strict guidelines regarding the appropriate time to terminate an experiment, and mentioned rabies vaccine testing in rodents as an example. In these tests, it becomes clear that a vaccine is effective when a circling behavior is observed in an animal, and the animal could be euthanized at that point to prevent further pain and distress. Dr. Goldberg felt that a procedure should be in place to prevent further pain and distress once the necessary information is obtained from test methods. Dr. Stokes agreed that more detailed guidelines are needed on this subject, and that this topic is addressed in a new guidance document being developed by the Organization for Economic Coordination and Development (OECD). Dr. Goldberg stated that the draft OECD Report on Humane Endpoints is expected to be available for comment on September 28, and that a Nominated Expert Meeting to review the comments and draft is scheduled in the Netherlands for November 19-20, 1998.

Dr. Theran commented that there is enthusiasm for ICCVAM in the animal welfare community. He asked if the LLNA can be used if its performance is similar to current guinea pig tests, or if the new method must show better performance to justify its use. Dr. Stokes replied that it would likely be used since the peer review panel concluded that the method's performance is at least as good as the guinea pig tests. He added that finding better alternatives, with respect to animal welfare considerations, is one of the primary goals of ICCVAM. Dr. Theran commented that this needs to be articulated more clearly in the goals statement.

Dr. Goldberg complimented ICCVAM on their progress and stated that while he had been somewhat skeptical about the probable success of the validation process, that clearly a very effective and successful process had been established. He asked Dr. Gerberick to provide insight into the strong and weak points of the review process from the perspective of a test sponsor. Dr. Gerberick indicated that he would address this issue during his presentation.

Dr. Rowan complimented all involved in the development of ICCVAM and NICEATM. He asked about the number of test method evaluations anticipated by ICCVAM. Dr. Stokes replied that NICEATM is at maximum capacity for its current base funding level with three test methods under review. Additional funding and staff would be required to carry out more than three peer reviews or workshops per year, but this can be accomplished through the NICEATM support contract with up to six additional reviews or workshops a year.

Dr. Neil Wilcox, Food and Drug Administration (FDA) stated that the FDA is concerned about the potential adverse impact of ICCVAM participation because of understaffing at FDA. However, due to the importance of this process, the FDA was committed to staying actively involved.

Peer Review Panel Report on the LLNA

Dr. Bailey, who served as a member of the Peer Review Panel (Panel) for the LLNA, presented an update on the LLNA test method review process and also summarized the conclusions of the Panel. The Panel concluded that the LLNA results, as submitted and supplemented by the Sponsors, demonstrated that the assay performed at least as well as currently accepted guinea pig

methods (Guinea Pig Maximization Test [GPMT]/Beuhler Assay [BA]) for the hazard identification of strong to moderate chemical sensitizing agents. The Panel also concluded that the LLNA offers advantages with respect to animal use refinement compared to conventional guinea pig methods in that it involves less pain and distress. The Panel recommended the LLNA could be used as a stand-alone alternative for contact sensitization hazard assessment, but recommended some protocol modifications. Following the presentation, Dr. Bailey opened the floor for questions.

Dr. Hurt asked if the LLNA could be used to determine if a compound is not a sensitizer. Dr. Bailey replied that the LLNA was validated as a stand-alone assay, and that a negative call in the assay meant that the chemical was not a sensitizer.

Dr. Montgomery commented that certain kinds of animal bedding have been shown to disrupt endocrine function, and asked whether the type of bedding was considered to be important for the LLNA. He also noted that the nomenclature for lymph node anatomy differs between the U.S. and Europe. He emphasized that this difference needs to be addressed. Dr. Bailey replied that the type of bedding had not been addressed, but he did not consider that standard laboratory bedding would have an adverse effect on the assay. He added that the Panel recommended that an illustration be added to the protocol indicating the location of the nodes draining the exposure site that are to be harvested. It was also recommended that, as a training method, known sensitizers should be used in pilot studies to induce proliferation so that skill can be gained in identifying the appropriate auricular node.

Dr. Montgomery expressed concern about multiple housing of animals in this assay, considering that the animals will groom each other, resulting in an oral dose as well as a dermal dose. Dr. Gerberick replied that the animals were individually housed in the U.S. studies and group-housed in the European studies. No difference in the results from the U.S. and European collaborators were identified. Thus, housing conditions did not seem to affect assay results.

Dr. Montgomery asked if there was a difference in results obtained using nodes pooled among animals compared to results obtained using individual animal data. Dr. Gerberick responded that the Panel concluded that individual animal response data should be collected to allow for statistical analysis in addition to calculation of the stimulation index (SI).

Dr. Goldberg asked if there was a meeting to discuss assay acceptance criteria before the Panel meeting. Dr. Stokes replied that evaluation guidance emphasizing the criteria was provided, but that there was not a preset standard for accepting or rejecting an assay.

LLNA Test Method Review Process

Ms. Denise Sailstad, ICCVAM Immunotoxicity Working Group (IWG) Co-Chair, presented an overview of the IWG perspective of the review process. She discussed the purpose and role of the IWG in the process, as well as contributions of the IWG. ICCVAM working groups are established as subcommittees to coordinate the test method peer review, provide input to the Sponsors, and to forward recommendations to agencies via ICCVAM; these recommendations are determined by the working group based on the peer review report. Specific challenges of this first working group included maintaining the focus of the review, developing new processes for ICCVAM, and facilitating communication between the Sponsor, NICEATM, ICCVAM, and the Panel.

Dr. Bailey then provided an update from the Panel perspective. He stated that the process was excellent in that clear objectives were provided to the panel, the process was well planned, and that information was thorough and received in a timely manner. He added that the sponsors responded well to requests, and that the panel worked well together.

Lastly, Dr. G. Frank Gerberick, Procter & Gamble, provided an overview of the LLNA process from the sponsor's perspective. He stated that industry is happy to see a process such as ICCVAM in place, and felt that the process, while demanding on the sponsor, was effective. Dr. Gerberick thanked ICCVAM and IWG representatives, as well as the panel and the Center, for their efforts. Following the presentations, the floor was opened for further discussion.

Dr. Goldberg asked for elaboration on the difference between standard operating procedures (SOPs) and protocols. Dr. Bailey replied that a protocol provides general guidance while an SOP provides the specific details necessary to conduct the test. He reiterated that the panel would have preferred to review SOPs rather than a protocol. Dr. Bailey also commented that a presentation by the LLNA Sponsor to show exactly how the process is performed would have been helpful to the Panel.

Dr. Goldberg suggested that names of future peer reviewers could be obtained by request from scientific societies as was done for the LLNA peer review panel. He also suggested that minimum submission criteria be established for future submissions.

Dr. Green requested information on how the various regulatory agencies proposed to respond once the Panel conclusions are submitted for consideration. Dr. Wilcox stated that the FDA has begun to establish procedures for reviewing ICCVAM results.

Dr. Karen Hamernik, EPA, also stated that the EPA is beginning to establish a process for evaluating ICCVAM results. She suggested that more EPA staff needed to participate in the working group earlier in the review process, and commented that EPA staff had questions regarding weak and non-sensitizers, the performance of the LLNA compared to currently used guinea pig assays, and the number of weak sensitizers evaluated.

Ms. Sailstad replied that there is a need to have confidence in the process and the competency of the Panel, and that considerable efforts were made to have information available to interested regulatory representatives. She also stated that the questions raised by EPA representatives had been addressed by the Panel. Dr. Lucier also stated that, for the process to work, the agencies needed to have confidence in the process.

Dr. Wilcox concluded that at the FDA, IWG participants were considered to be the key to regulatory acceptance. The FDA is comprised of seven organizational centers with different mandates. Thus, acceptance is expected to vary between centers. He also requested information on the meaning of the "spirit" of Good Laboratory Practices (GLPs). Dr. Stokes mentioned that more clarity will be added to the submission guidelines to address this issue.

Dr. Hurt stated that there is a need to recognize the centrality of the ICCVAM Working Group in the process and to ensure that all constituents are adequately represented. She also recommended that the sponsor should provide a proposed test method guideline similar to that used by the EPA and OECD.

Dr. Green asked how ICCVAM will respond to the Panel request for a quality assurance audit and he asked what ICCVAM would do if the data were found to be inadequate. Dr. Green also asked if there is a process for evaluating the Panel recommendations. Dr. Stokes replied that if the audit revealed erroneous data, that it would likely be deleted from the analysis. In addition, the IWG will be asked to review the Panel recommendations, and to forward their position on these recommendations to ICCVAM for consideration.

Dr. Stitzel asked how recommended changes or suggestions will be incorporated into the final report. Dr. Stokes stated that these recommendations will be taken to ICCVAM for consideration, and then will be forwarded to agencies.

NTP Center/ICCVAM Website

Mr. Patrick Herron, NICEATM Web Operations Manager, gave a presentation on the NICEATM and ICCVAM website, and announced that the website could be accessed at the following address (**http://iccvam.niehs.nih.gov/**). He then reviewed some of the information available on the site, such as the Submission Guidelines, Federal Register Notices for upcoming meetings and publication releases, and the ICCVAM report entitled, "Validation and Regulatory Acceptance of Toxicological Test Methods," NIH Publication No. 97-3981.

Update on the EPA Endocrine Disrupter Screening and Testing Initiative

Dr. Maciorowski, EPA, presented an update on the EPA Endocrine Disrupter Screening and Testing Initiative. He presented background information on the justifications for and against endocrine disrupters testing, and stated that the basis for public concern related to the scientifically plausible hypothesis that wildlife incidents have occurred, that there are epidemiological and experimental studies available, and that uncertainties exist. He also reviewed EPA's Food Quality Protection Act mandates and recommendations of the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC). EDSTAC recommendations include the use of automated robotic prescreens to set priorities; a relational database to set priorities and track data; eight screening assays to detect potential estrogenic effects; and five tests using mammals, birds, fish, frogs, and shrimp to verify and characterize adverse effects. The proposed screening assays include the Estrogen Receptor Binding or Reporter Gene Assay, the Androgen Receptor Binding or Reporter Gene Assay, the Steroidogenesis Assay using Minced Testis, the Rodent Three-Day Uterotropic Assay, the Rodent 20-Day Pubertal Female Assay with Thyroid, the Rodent Five- to Seven-Day Hershberger Assay, the Frog Metamorphosis Assay, and the Fish Gonadal Recrudescence Assay. They also recommended validation of all the screens and tests prior to regulatory implementation. The floor was then opened for discussion.

Dr. Lucier suggested that binding/reporter gene assay experts and those familiar with receptor-mediated assays serve in the working group. Dr. Maciorowski replied that there were three working groups in place: one for *in vitro* tests, one for mammalian *in vivo* tests, and a third for non-mammalian *in vivo* tests.

Dr. Stitzel asked about the "gold standard" for chemicals that can be used in such assays and stated that there is a need to determine what will be called positive or negative.

Dr. Rowan asked whether any of these assays were considered to be prevalidated. Dr. Lucier replied that the uterotropic assay was considered to be prevalidated, but generally, most others were not.

Dr. Stitzel felt there was a need for the validation committee to promote inter-laboratory validation studies.

Dr. Lucier asked if there was confidence in the androgen/estrogen reporter gene assay database. Dr. Denison stated that reporter problems stemmed from an elevated background and that information from receptor binding and reporter assay combinations was needed.

Dr. Rowan asked if the entire battery of tests were necessary or if there is redundancy in the results obtained. Dr. Lucier replied that it is difficult to define endocrine disrupters, and that a variety of tests are needed to capture the exact mechanisms. Dr. Maciorowski further stated that EDSTAC

tried to determine how to handle results within the battery, and that the validation process would result in data that could be used to identify redundant tests.

Dr. Goldberg stated that the number of compounds that need to be tested for endocrine disruption is formidable and that the amount of time allotted to EPA by Congress does not seem sufficient.

Dr. Zeiger also felt that there was a need to be cautious about the amount of time that will be required because of the large volume of chemicals.

Ocular Irritancy Testing Methods: International Life Sciences Institute (ILSI) Task Force Report

Dr. Stitzel provided an update on recommendations for improving ocular irritancy testing methods developed by an ILSI Task Force. She provided background information on ILSI and its Technical Committee on Alternatives to Animal Testing. Dr. Stitzel then provided an overview of the expert review of eye irritation testing methods, including the conclusions and the proposed classification scheme. The expert panel on ocular irritation concluded that the rabbit Draize test is not the appropriate "gold standard"; evaluation of ocular irritation should be based on human experience; the focus should be on developing mechanistically based *in vitro* tests that predict initial human injury; and a new injury classification scheme is needed to standardize assessment of the human eye response to irritating materials.

Unrelieved Pain and Distress in Toxicology Testing: Updated on the HSUS Initiative

Dr. Rowan presented an update on the HSUS initiative to eliminate unrelieved pain and distress in toxicology testing, focusing on the refinement of techniques in research and testing. As part of the campaign, the HSUS has prepared an expert group report aimed at specialized workshops in testing methods for toxicology, immunology, and infectious diseases. Dr. Rowan explained that the HSUS is hosting pain and distress campaign workshops as part of the outreach initiative. Following the presentation, he opened the floor for questions.

Dr. Goldberg stated that a National Academy of Sciences (NAS) workshop on pain and distress would take place on November 2-3, 1998. The NRC Institute for Laboratory Animal Research is preparing a report on monoclonal antibody production, with a focus on avoiding animal use unless necessary.

Dr. Stokes stated that there were two upcoming meetings related to pain and distress: an OECD-nominated expert workshop scheduled for November 19-20, 1998, and a European Center for the Validation of Alternative Methods (ECVAM) sponsored workshop scheduled for November 23-25, 1998.

Closing Comments

Dr. Rowan asked the status of the ICCVAM review of Corrositex. Dr. Stokes stated that the peer review meeting was scheduled for January 21, 1999.

Dr. Green concluded that sweeping, radical changes in the ICCVAM process shouldn't be made but that minor modifications may be necessary to improve efficiency. He advised waiting until one more assay has been reviewed before evaluating the process and suggesting changes.

Dr. Theran stated that the animal protection community was pleased with the progress of ICCVAM thus far.

Dr. Goldberg further stated that implementation of the ICCVAM recommendations by the agencies is the critical step for completing the regulatory acceptance process.

Adjournment
The next meeting was scheduled for March 4, 1999, and the meeting was adjourned at 4:15 p.m.